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PATHOTYPE ANALYSIS OF ESCHERICHIA COLI ISOLATED FROM NEWBORN AND POST-WEANING PIGS WITH DIARRHEA

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FINAL REPORT (ADF PROJECT 19990264)

Pathotype analysis of *Escherichia coli* isolated from newborn and post-weaning pigs with diarrhea.

(a) ABSTRACT

To determine the prevalence of *E. coli* pathotypes in diarrheic piglets, *E. coli* strains was isolated from 170 diarrheic and 120 healthy piglets. The isolates were tested for the presence of F4, F5, F6, F18, F41 fimbriae, for EAE (*E. coli* attaching and effacing) and AIDA (adhesin involved in diffuse adherence) factors, and for STa, STb, EAST-1, LT, Stx1 and Stx2 toxins, using PCR and DNA/DNA hybridization. All isolates were tested for antibiotic resistance against 12 drugs. Seventeen pathotypes, accounting for 40.6% of the strains, were isolated from diarrheic piglets. The main pathotypes included EAST-1 (13.5%), F4/STb/LT/EAST-1 (6.5%), AIDA/STb/EAST-1 (4.7%), F5/STa (2.9%), EAE/EAST-1 (2.9%) and F18/AIDA (2.9%). Minor pathotypes accounted for 7.2% of the isolates. Only 3 pathotypes EAE (11.7%), EAST-1 (10.8%) and EAE/EAST-1 (3.3%) were detected from healthy piglets. Overall, 23.5% of diarrhea cases were attributed to *E. coli* of which 5.3% appeared to be associated with a distinctive enteroaggregative *E. coli* (EaggEC) pathotype AIDA/STb/EAST-1 and 3.6% of unclassified pathotype, isolated only from diarrheic piglets. All isolates from this pathotype were sensitive to enrofloxacin (ENO), gentamicin (GM), neomycin (NE) and sulfamethoxazole-trimethoprim (SXT). Our data showed that at least 22.5% of piglet enteric colibacillosis studied were associated with a distinctive EaggEC pathotype and treatment of related cases may include ENO, GM, NE or SXT.

(b) SUMMARY

Enteritis or inflammation of the intestinal tract, is an important cause of morbidity and mortality in livestock in general, and is one of the most common diseases of suckling and post-weaning pigs worldwide (**Alexander, T. J. L., and Hampson, D. J.** 1994. In C. L. Gyles [ed.]. p.151 and p.171. CAB International, UK). It is believed that among swine pre-weaning diseases, diarrhea has the highest morbidity and represents approximately 10.8% of pre-weaning mortality (**Tubbs, R. C.** et al. 1993. Swine Health Prod. 1:21). The infection can occur at any time during suckling but the highest incidence of disease occurs during the first 3 to 5 days of life. Enterotoxigenic *E. coli* has been commonly incriminated as one of the most important etiological agent of the disease (**Kwon, D.** et al. 1999. J. Vet. Diagn. Invest. 11:146). During the last few years, we have observed that the etiological agent of a large numbers of diarrhea cases in young pigs was not determined, using standard routine laboratory techniques (**Ngeleka, M.** 1999. Prairie Diagnostic Services newsletter, 1:2). In some cases the use of commercial fimbriae-based *E. coli* vaccines such as F4, F5, F6, and F41 vaccines on pregnant sows does not seem to protect the offspring from diarrhea. Antibiotic therapy seems to cure the infection only in a certain number of cases, but in other diarrhea cases, the emergence of multi-resistant *E. coli* isolates renders this practice inefficient. Based on clinical and post-mortem examinations, and laboratory testing on some of these diarrhea cases, there is strong evidence that *E. coli* is probably the etiological agent. However, it is not known whether these cases are caused by emerging *E. coli* strains or are due to changes in the genome material of classical enterotoxigenic *E. coli* strains.

The main objective of the project was to determine whether or not newborn and post-weaning pig diarrhea is caused by emerging *E. coli* strains or by variants of classical enterotoxigenic *E. coli* strains.

E. coli strains were isolated from 170 diarrheic and 120 healthy piglets. The isolates were identified using standard biochemical tests for identification of enterobacteria (**Quin, P. J.** 1994. Wolfe Publishing. Mosky-year Book Europe Limited. Spain). The *E. coli* isolates were tested for the presence of F4, F5, F6, F18, F41 fimbriae, for EAE (*E. coli* attaching and effacing) and for AIDA (adhesin involved in diffuse adherence) factors. In addition, the isolates were tested for the heat stable enterotoxins (STa, STb, EAST-1), heat labile enterotoxin (LT) and Shiga-like toxin 1 and 2 (Stx1 and Stx2), using Polymerase Chain

Reaction (PCR) and DNA/DNA hybridization. Furthermore, all isolates were tested for sensitivity against 12 antimicrobial drugs such as ampicillin, apramycin, carbadox, ceftiofur, enrofloxacin, gentamicin, neomycin, spectinomycin, tetracycline, tiamulin, tilmicosin and trimethoprim-sulfamethoxazole.

Randomly selected specimens from piglets with diarrhea were tested for common virus- or parasite-inducing diarrhea in piglets and processed for histopathology analyses using standard procedures for Hematoxilin and Eosin.

Progressive evaluation of the project

- **Evaluation of the project after 38 accessions**

Virology and parasitology analysis performed on 38 clinical cases showed that only 5.2% of cases were positive for Rotavirus, the remaining cases were negative for Coronavirus and Transmissible gastro-enteritis virus. On the other hand, only 2% of cases yielded *Isospora suis*. These results suggest that viruses and other parasites are probably involved in young pigs diarrhea at a lower level (see result section below). Therefore, we have decided to discontinue virology and parasitology testing, and to focus only on *E. coli*, the main objective of the project.

- **Evaluation of the project after 100 accessions**

After analysis of 100 specimens from which *E. coli* was isolated, and based on new information acquired during the project, we have decided to introduce the DNA-DNA hybridization analysis on all the isolates and to test all the isolates for O-serotyping. To accommodate the budget, this was done by reducing the number of specimens to be processed from both diarrheic and healthy piglets to a maximum of 300 (see more details under project activities, technical report (c) below.

RESULTS

A total of 170 cases from newborn, young pigs and post-weaning pigs with diarrhea, and 120 fecal swabs from healthy looking piglets up to a week of age were collected for the study.

Of the 170 *E. coli* isolates from piglets with diarrhea, 17 different pathotypes, accounting for 40.6% of the isolates, were recovered. The main pathotypes were EAST-1 (13.5%), F4/STb/LT/EAST-1 (6.5%), AIDA/STb/EAST-1 (4.7%), F5/STa (2.9%), EAE/EAST-1 (2.9%) and F18/AIDA (2.9%). Minor pathotypes included STb (1.2%), and STb/EAST-1, STb/LT/EAST-1, LT, F4/LT, F4/STa, F5/STa/STb, F5/F6/STa, AIDA/STb, AIDA/EAE/EAST-1 and AIDA, accounting for 0.6% each.

Of the 120 *E. coli* isolates from healthy pigs, only 3 pathotypes including EAE (11.7%), EAST-1 (10.8%) and EAE/EAST-1 (3.3%) were recovered.

Overall, 23.5% of all diarrhea cases were attributed to *E. coli*: enterotoxigenic *E. coli* (ETEC) accounted for 11.7%, enteropathogenic *E. coli* (EPEC) for 2.9% and unclassified pathotypes for 3.6%. However, 5.3% of the isolates appeared to be distinctive enteroaggregative *E. coli* (EaggEC) which included AIDA/STb/EAST-1 and AIDA/STb pathotypes isolated only from of piglets with diarrhea.

In general, the isolates from diseased pigs had higher level of resistance against the tested antimicrobial agents, as compared to isolates from healthy pigs. However, no resistance was detected against enrofloxacin and low level of resistance was observed against ceftiofur, apramycin and gentamicin. Among ETEC isolates, a high level of resistance against most of the tested antimicrobial drugs was observed. Approximately 5% of isolates were resistant to carbadox and 10% to gentamicin, however, all the isolates were sensitive to ceftiofur and enrofloxacin. Similarly, high level of resistance was observed among the AIDA/STb/EAST-1 or AIDA/STb pathotypes isolates of EaggEC, but all the isolates were sentivive to enrofloxacin, gentamicin, neomycin and trimethroprim-sulfametoxazole.

Beside *E. coli* infection, some selected cases from piglets with diarrhea yielded *Salmonella* spp., Coronavirus, Rotavirus, Transmissible gastro-enteritis virus and *Isospora suis*.

Histopathology done on selected clinical cases involving classical enterotoxigenic *E. coli* showed the characteristic attachment of bacteria to the intestine epithelial cells. However, examination of some clinical cases involving the EaggEC pathotype from diarrheic piglets was not conclusive, suggesting that such examination should probably be done on tissues collected from animals

experimentally infected with appropriate isolates. A research project on the subject is underway with Drs. D. Middleton and J. Pritchard from the Department of Pathology at the Western College of Veterinary Medicine with our collaboration.

(c) TECHNICAL REPORT

Project activities: The project account was set up in April 2000. The project started in May 1, 2000. By May 30, 2000 the primers were designed and the first specimens were analyzed on June 3, 2000. PCR and DNA/DNA hybridization analysis of all selected isolates have been completed. O-serotyping of the isolates is being performed in a reference laboratory and results will be part of a manuscript for publication, later this year.

An abstract (attached) has been submitted for a conference presentation in November 2001, at the American Association of Veterinary Laboratory Diagnosticians in Hershey, Pennsylvania. At that time, a manuscript should be ready for possible publication in a refereed journal.

Discussion of results and conclusions

Our data showed that 40.6% of the *E. coli* isolated from diarrhea cases could be assigned a pathotype. With the exception of EAST-1 (23 isolates, 13.5%), F18/AIDA (5 isolates, 2.9%) and AIDA (1 isolate, 0.6%) pathotypes; 23.5% of all diarrhea cases were attributed to *E. coli* infection. Of these, 11.7% of cases involved a classical ETEC (F4, F5 or F6-positive *E. coli*), 2.9% a classical EPEC (EAE-positive *E. coli*) and 3.6% unclassified pathotype. However, 5.3% of the cases appeared to involve distinctive EaggEC pathotypes AIDA/STb/EAST-1 or AIDA/STb only isolated from diarrheic piglets. EAST-1 pathotype was recovered in high numbers (13.5%) from pigs with diarrhea, but also from healthy pigs, in relatively similar numbers (10.8%), suggesting that expression of EAST-1 pathotype alone may not be a significant virulence marker. In addition, AIDA/F18 (2.9%) and AIDA (0.6%) pathotypes did not seem to be associated with any enterotoxin, thus making the role of these pathotypes in diarrhea uncertain.

EAST-1 toxin is a heat-stable enterotoxin described in *E. coli* isolated from cases of enteritis in children (Vila, J., et al. J. Med. Microbiol. 47:889-891, 1998), and has been reported recently in piglets with diarrhea (Choi, C et al. J. Vet. Diagn. Invest 13:26-29, 2001). This last study suggests that EAST-1 enterotoxin may represent an important virulence determinant of *E. coli*

causing diarrhea in piglets. However, as found in our study, recovery of EAST-1 positive *E. coli* isolates from piglets with diarrhea and also from healthy piglets in similar numbers makes the role of this enterotoxin in young pigs diarrhea uncertain.

AIDA is a plasmid-encoded outer membrane protein involved in diffuse adherence phenotype of EaggEC isolated from infants with persistent episode of diarrhea (Benz, I et al. 1989. Infect. Immun. 57:1506-1511). This factor has not been fully investigated in the porcine. However, isolation of AIDA-positive *E. coli* strains, in association with other enterotoxin such as STb, only from diseased piglets suggests a role of this protein in production of diarrhea.

In general bacterial virulence is a multi-factorial feature and usually includes more than one virulence determinant. These factors include fimbriae (e.g. F4, F5, F6) and enterotoxins (e.g. STa, STb, LT) for full pathogenicity, as detected among the ETEC strains isolated in this study. With the exception of EAE/EAST-1 pathotype detected in diarrheic piglets, but also from healthy piglets, isolation of AIDA/STb/EAST-1 or AIDA/STb pathotypes only from diarrheic piglets suggest a potential role of such pathotypes in porcine diarrhea. We have evidence that *E. coli* isolates positive for these pathotypes are significant pathogens of diarrhea in piglets, as demonstrated in a project conducted by Dr. D. Middleton (Department of Veterinary Pathology) with our collaboration.

In fact, Dr Middleton preliminary results on experimental infection of colostrum deprived newborn pigs showed that piglets inoculated with either AIDA/STb/EAST-1 or AIDA/STb had diarrhea. In contrast piglets inoculated with an isolate positive only for EAST-1 or negative controls inoculated with 0.1% peptone water did develop diarrhea. This suggests that AIDA/STb/EAST-1 or AIDA/STb *E. coli* pathotypes of EaggEC may be associated with enteric colibacillosis, rather than EAST-1 pathotype.

The antibiotic resistance profile showed that more isolates from diarrheic pigs were resistant against the tested antimicrobial drugs, as compared to the isolates from healthy piglets. In general, all the isolates were sensitive to enrofloxacin, low level of resistance was detected against ceftiofur, apramycin and gentamicin, and moderate level of resistance against carbadox and neomycin. However, with the last antimicrobial, high numbers of isolates were of intermediate sensitivity. High level of resistance was detected against trimethoprin-sulfametoxazole, ampicillin, and spectinomycin. All the isolates (more than 90%) were resistant to tetracycline, tiamulin and tilmicosin. The level of antibiotic resistance varies among the major

pathotypes, but all the ETEC isolates were sensitive to ceftiofur and enrofloxacin, and all EaggEC isolates of the AIDA/STb/EAST-1 or AIDA/STb pathotypes were sensitive to enrofloxacin, gentamicin, neomycin and trimethroprim-sulfametoxazole. Recovery of high numbers of resistant isolates from diarrheic piglets, as compared to isolates from healthy piglets suggest that resistance genes in these isolates be probably linked to some virulence determinants. Overall, the best choice for treatment of *E. coli* enteritis in piglets with diarrhea, irrespective of the etiologic pathotype, appears enrofloxacin, ceftiofur, apramycin or gentamicin.

We have demonstrated that 23.5% of diarrhea cases in our study, were attributed to *E. coli* and that 22.5% of these cases are caused by distinctive pathotypes of EaggEC. Isolation of approximately 2.9% of unclassified pathotype is also of interest.

Our findings suggest that in presence of infection caused by isolates of the EAggEC pathotypes or of the unclassified pathotype, routine diagnostic technique using slide agglutination method will probably not allow detection of such pathogens. Furthermore, our results suggest that a vaccination program with classical fimbrial-based vaccines may not be efficacious for the prevention of related diarrhea cases.

Based on our results, it will be necessary to determine the pathogenesis of the distinctive EaggEC pathotypes and also to investigate more on the unclassified pathotype. Such studies are necessary for the evaluation of current vaccination program and subsequently for a better protection of piglets against related enteric collibacillosis.

(e) No major equipment was purchased with project funds.

(f) An abstract for an oral presentation to the American Association of Veterinary Laboratory Diagnosticians conference in November 2001 is attached. A copy of a final manuscript will be forwarded later.

(g) No photos were taken.

(h): Saskatchewan Agricultural development Fund and the Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan names will be mentioned in the Acknowledgment section of all presentations and publications.